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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of
Serial No. 10/539,234
Shirou Maeda et al.
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DECLARATION (1) UNDER 37 CFR 1.132

Honorable Commissioner of Patents and Trademarks

Sir,

I, Shirou Maeda declare that:

I am a citizen of Japan and a resident of Nagaokakyo-shi, Kyoto Prefecture, Japan;

I graduated from a graduate school, Master's Course, Faculty of Science, The Osaka City University, Japan in 1967;

I received the Doctor degree on the study of "Studies on the Synthesis of Bioactive Lignans by Oxidative Coupling Reaction" from The Osaka City University, Japan in 1995;

I have been an employee of Hamari Chemicals, Ltd. Japan, since October of 2000 up to this time;

I have been engaged in research at Core Technology Laboratory;

I am one of inventors of the above-identified patent application;

The experiments set out below were conducted under my supervision and direction.

EXPERIMENTS

Experiment 1 Preparation of test compounds

(1) 3,6-Dihydro-6,6-dimethyl-4-decylamino-2-(4'-methoxybenzylamino)-1,3,5-triazine hydrochloride (the compound of Example 1 in the specification)

80 ml of methanol, 120 ml of acetone and 0.1 ml of concentrated hydrochloric acid were added to 2.0 g (4.6 mmol) of N^1-(4-methoxybenzyl)-N^5-decyl-biguinide dihydrochloride, and the mixture was refluxed for 21 hours. After removal of the solvent by evaporation under reduced pressure, the residue was dissolved in a 80% aqueous acetonitrile solution, and the solvent was removed by evaporation under reduced pressure. The residue was purified by subjecting to silica gel column chromatography (elution with a mixture of chloroform/ethanol/acetic acid (9:0.5:9.5) to obtain 1.7 g of a colorless resinous solid.

^{1}H-NMR($CDCl_3$) δ: 0.87(3H,t,J=7Hz,CH_3), 1.1-1.6(16H,m), 1.40(6H,s,$(CH_3)_2C$), 3.28(2H,br dt-like,$NHC\underline{H}_2$), 3.77(3H,s,CH_3O), 4.45(2H,d,J = 5Hz,$ArC\underline{H}_2NH$), 6.81(2H,d,J = 8Hz,ArH), 7.11(1H,br t-like,$N\underline{H}CH_2$), 7.19(2H,d,J = 8Hz,ArH), 7.45(1H,br t-like,$ArCH_2\underline{N}H$), 8.47,8.60(each 1H,br s,NH,NH^+).

By ^{1}H-^{1}H COSY, coupling was recognized between $NHC\underline{H}_2$(δ: 3.28), $N\underline{H}CH_2$(δ: 7.11), $ArC\underline{H}_2NH$(δ: 4.45) and $ArCH_2NH$(δ: 7.45) signals. In addition, no coupling was recognized between triazine ring NH and NH^+(δ: 8.47,8.60) signals and other proton.

(2) 3,6-Dihydro-6,6-dimethyl-4-decylamino-2-benzylamino-1,3,5-triazine methanesulfonate (the compound of Example 2 in the specification)

To a solution of 11.0 g (27.2 mmol) of N^1-benzyl-N^5-decyl-biguanide dihydrochloride in 150 ml of methanol was added 16 ml of 5N aqueous sodium hydroxide, and the mixture was stirred at 60°C for 30 minutes. After removal of the solvent by evaporation under reduced pressure, the residue was extracted with chloroform. The extract was washed with water, the solvent was removed by evaporation under reduced pressure, and 100 ml of acetone and 16 g (19.0 mmol) of piperidine were added to the residue. The mixture was refluxed for 17 hours, and then concentrated under reduced pressure to remove the solvent. The residue was washed with water, and sufficiently dried under reduced pressure to obtain 10.0 g of a colorless resinous solid. Then, 2.5 g (6.7 mmol) of the above solid was dissolved in 50 ml of acetone, and 16 g (16.7 mmol) of methanesulfonic acid was added thereto. The solvent was evaporated off under reduced pressure to give a residue, which was dissolved in 70% aqueous acetonitrile. The solvent was removed by evaporation under reduced pressure, and the residue was purified by silica gel column chromatography (elution with a mixture of chloroform and methanol (9:1.5)), and ether was added for crystallization, yielding 2.6 g of colorless crystals having a melting point of not higher than 50°C.

^{1}H-NMR($CDCl_3$) δ: 0.88(3H,t,J=7Hz,CH_3), 1.1-1.6(16H,m), 1.40(6H,s,$(CH_3)_2C$), 2.76(3H,s,$\underline{CH_3}SO_3^-$), 3.22(2H,br dt-like,NH$\underline{CH_2}$), 4.50(2H,d,J = 6Hz,Ar$\underline{CH_2}$NH), 7.16(1H,br t-like,NH), 7.2-7.3(5H,m,ArH), 7.60(1H,t,J = 6Hz,$\underline{NH}$), 7.96,8.09(each 1H,br s,NH,NH^+).

(3) 3,6-Dihydro-6,6-dimethyl-4-decylamino-2-(4'-methoxyphenethylamino)-1,3,5-triazine hydrochloride (the compound of Example 4 in the specification)

125 ml of methanol, 80 ml of acetone and 0.2 ml of concentrated

hydrochloric acid were added to 2.0 g (4.5 mmol) of N^1-(4-methoxyphenethyl)-N^5-decyl-biguanide dihydrochloride. The mixture was refluxed for 24 hours, and the solvent was distilled off under reduced pressure. The residue was purified by silica gel column chromatography (elution with a mixture of chloroform/methanol/acetic acid (8:0.6:0.6)), and dissolved in 70% aqueous acetonitrile. The solvent was distilled off under reduced pressure, followed by sufficient drying under reduced pressure to obtain 2.1 g of a colorless resinous solid.

^{1}H-NMR($CDCl_3$) δ: 0.87(3H,t,J=7Hz,CH_3), 1.12-1.6(16H,m), 1.43(6H,s,$(CH_3)_2C$), 2.80(2H,t,J = 7Hz,Ar$\underline{CH_2}CH_2NH$), 3.33(2H,br dt-like,NH$\underline{CH_2}$), 3.51(2H,br dt-like,Ar$CH_2\underline{CH_2}NH$), 3.77(3H,s,CH_3O), 6.82(2H,d,J = 9Hz,ArH), 7.11(2H,d,J = 9Hz,ArH), 7.08-7.16(1H,overlap,N$\underline{H}CH_2$), 7.21(1H,br t-like,Ar$CH_2CH_2N\underline{H}$), 8.49,8.51(each 1H,br s,NH,NH^+).

By ^{1}H-^{1}H COSY, coupling was recognized between NHC$\underline{H_2}$(δ: 3.33) and N$\underline{H}CH_2$(δ: 7.08-7.16), Ar$\underline{CH_2}CH_2NH$(δ: 2.80) and Ar$CH_2\underline{CH_2}NH$(δ: 3.51), Ar$CH_2\underline{CH_2}NH$ (δ: 3.51) and Ar$CH_2CH_2N\underline{H}$(δ: 7.21) signals. In addition, no coupling was recognized between triazine ring NH and NH^+ signals (δ: 8.49, 8.51) and other proton.

(4) 4-Octylamino-3,6-dihydro-6,6-dimethyl-2-(4'-methylbenzylamino)-1,3,5-triazine (the compound of Example 37 in the specification)

40 ml of methanol, 80 ml of acetone and 16 ml (16.2 mmol) of piperidine were added to 3.0 g (7.7 mmol) of N^1-(4-methylbenzyl)-N^5-octyl-biguanide dihydrochloride, and the mixture was refluxed for 23 hours. The solvent was distilled off under reduced pressure, and the residue was purified by silica gel column

chromatography (elution with a mixture of chloroform, methanol and acetic acid (9:0.5:0.5)). 50 ml of ethanol and 50 ml of water were added to the resulting colorless resinous solid, and the pH of the mixture was adjusted to 11 to 12 with 5N sodium hydroxide. The mixture was refluxed for 1 hour, and the solvent was distilled off under reduced pressure. The residue was washed with water, and dried well under reduced pressure to obtain 1.3 g of a colorless solid.
^{1}H-NMR($CDCl_3$) δ: 0.87(3H,t,J=7Hz,CH_3), 1.1-1.6(12H,m), 1.33(6H,s,$(CH_3)_2C$), 2.31(3H,s,$ArCH_3$), 3.16(2H,t,J = 7Hz,$NHC\underline{H}_2$), 4.36(2H,br s,$ArCH_2$), 7.09(2H,d,J = 8Hz,ArH), 7.18(2H,d,J= 8Hz,ArH).

(5) 3,6-Dihydro-6,6-dimethyl-4-decylamino-2-(4'-methoxybenzylamino)-1,3,5-triazine methanesulfonate (the compound of Example 38 (2) in the specification)

55 ml of 5N aqueous sodium hydroxide was added to 40 g (92.1 mmol) of N^1-(4-methoxybenzyl)-N^5-decylbiguanide dihydrochloride in 400 ml of methanol, and the mixture was stirred at 60°C for 30 minutes. The solvent was distilled off under reduced pressure, and the residue was extracted with chloroform. The extract was washed with water, and the solvent was distilled off under reduced pressure to give a residue, to which were added 450 ml of acetone, 150 ml of methanol and 6.4 ml (46.6 mmol) of piperidine. The mixture was refluxed for 15 hours, and the solvent was distilled off under reduced pressure. The residue was washed with water while stirring, dissolved in ethyl acetate, and washed with water. After removal of the solvent by evaporation under reduced pressure, the residue was dried under reduced pressure to obtain 37 g of a colorless solid (1). Then, 2.3 g (5.9 mmol) of the solid was dissolved in 100 ml of methanol, and 169 g (19.7 mmol) of methanesulfonic acid was added thereto. The

solvent was distilled off under reduced pressure, and the residue was dissolved in 70% aqueous acetonitrile. The solvent was distilled off under reduced pressure, and the residue was purified by silica gel column chromatography (elution with a mixture of chloroform and methanol (9:1.5)). Crystallization took place upon addition of ether to obtain 1.9 g of colorless crystals having a melting point of 56 to 58°C.

^{1}H-NMR($CDCl_3$) δ: 0.87(3H,t,J=7Hz,CH_3), 1.0-1.6(16H,m), 1.39(6H,s,$(CH_3)_2C$), 2.75(3H,s,$\underline{CH_3}SO_3^-$), 3.28(2H,br dt-like,NH$\underline{CH_2}$), 3.78(3H,s,CH_3O), 4.43(2H,d,J = 6Hz,ArCH_2), 6.82(2H,d,J = 9Hz,ArH), 7.1-7.3(1H,over lap,NH), 7.20(2H,d,J = 9Hz,ArH), 7.53(1H,t,J = 6Hz,N$\underline{H}CH_2$), 7.92,8.01(1H,br s,NH,NH^+).

(6) 3,6-Dihydro-6,6-dimethyl-4-nonylamino-2-benzylamino-1,3,5-triazine acetate (the compound of Example 48 in the specification)

300 ml of methanol, 250 ml of acetone and 1.2 ml of concentrated hydrochloric acid were added to 18.2 g (46.6 mmol) of N^1-benzyl-N^5-nonyl-biguanide dihydrochloride. The mixture was refluxed for 22 hours, and the solvent was distilled off under reduced pressure. The residue was dissolved in 300 ml of ethanol, and to the solution were added 200 ml of water and 18 ml of 5N aqueous sodium hydroxide. The mixture was refluxed for 1.5 hours, concentrated under reduced pressure, and extracted with ethyl acetate. The extract was washed with water, and the solvent was distilled off under reduced pressure to obtain 18.9 g of colorless crystals.

3.0 g of colorless crystals were recrystallized from ethanol/ether to obtain 1.9 g of colorless crystals having a melting point of 99 to 102°C.

^{1}H-NMR($CDCl_3$) δ: 0.87(3H,t,J=7Hz,CH_3), 1.0-1.4(12H,m),

1.31(6H,s,$(CH_3)_2C$), 1.44(2H,m,$NHCH_2\underline{CH_2}$), 1.92(3H,s,$\underline{CH_3}COOH$), 3.22(2H,br dt-like,$NH\underline{CH_2}CH_2$), 4.48(2H,d,J=5Hz,$ArCH_2$), 7.2-7.3(5H,m,ArH), 8.16,8.68(each 1H,br t-like,NH), 9.09,9.25(each 1H,br s,NH,NH^+).

(7) 3,6-Dihydro-6,6-dimethyl-4-nonylamino-2-benzylamino-1,3,5-triazine hydrobromide (the compound of Example 49 in the specification)

5.1 g (13.6 mmol) of colorless crystals of Working Example 48 were dissolved in 20 ml of 30% aqueous acetonitrile, and to the solution was added 5 ml of 47% hydrobromic acid. The mixture was cooled, and the resulting precipitated crystals were filtered, and recrystallized from 70% aqueous acetonitrile to obtain 5.0 g of colorless crystals having a melting point of 91 to 93°C.

^{1}H-NMR($CDCl_3$) δ: 0.87(3H,t,J=7Hz,CH_3), 1.0-1.6(14H,m), 1.43(6H,s,$(CH_3)_2C$), 3.26(2H,br dt-like,$NHC\underline{H_2}$), 4.52(2H,d,J=6Hz,$ArCH_2$), 6.87(1H,br t-like,NH), 7.1-7.4(6H,m,ArH,NH), 8.16,8.31(each 1H,br s,NH,NH^+).

(8) 3,6-Dihydro-6,6-dimethyl-4-nonylamino-2-phenethylamino-1,3,5-triazine acetate (the compound of Example 56 in the specification)

350 ml of methanol, 350 ml of acetone and 1.9 ml of concentrated hydrochloric acid were added to 31.0 g (76.7 mmol) of N^1-phenethyl-N^5-nonyl-biguanide dihydrochloride. The mixture was refluxed for 16 hours, and the solvent was distilled off under reduced pressure. The residue was dissolved in 350 ml of ethanol, and to the solution were added 250 ml of water and 31 ml of 5N sodium hydroxide. The mixture was refluxed for 1.5 hours, concentrated under reduced

pressure, and extracted with ethyl acetate. The extract was washed with water, and the solvent was distilled off under reduced pressure. The residue was recrystallized from ether to obtain 16.3 g of colorless crystals having a melting point of 72 to 76°C.

^{1}H-NMR($CDCl_3$) δ: 0.86(3H,t,J=7Hz,CH_3), 1.1-1.4(12H,m), 1.34(6H,s,$(CH_3)_2C$), 1.54(2H,m,$NHCH_2\underline{CH_2}$), 1.93(3H,s,$\underline{CH_3}COO^-$), 2.84(2H,t,J=6Hz,$ArCH_2\underline{CH_2}NH$), 3.31(2H,br dt-like,$NHCH_2\underline{CH_2}$), 3.53(2H,br dt-like,$ArCH_2\underline{CH_2}NH$), 7.1-7.3(5H,m,ArH), 8.15,8.29(each 1H,m,NH), 9.12,9.23(each 1H,br s,NH,NH^+).

(9) 4-Octylamino-3,6-dihydro-6,6-dimethyl-2-(4'-methylbenzylamino)-1,3,5-triazine acetate (the compound of Example 58 in the specification)

300 ml of methanol, 180 ml of acetone and 1.2 ml of concentrated hydrochloric acid were added to 18.0 g (46.1 mmol) of N^1-4-methylbenzyl-N^5-octyl-biguanide dihydrochloride. The mixture was refluxed for 24 hours, and the solvent was distilled off under reduced pressure. The residue was dissolved in 200 ml of ethanol, and to the solution were added 140 ml of water and 18.5 ml of 5N aqueous sodium hydroxide. The mixture was refluxed for 1.5 hours, concentrated under reduced pressure, and extracted with ethyl acetate. The extract was washed with water, and the solvent was distilled off under reduced pressure. The residue was dissolved in ether, and cooled. The resulting precipitated crystals were filtered off, and recrystallized from ethanol/ether to obtain 10.1 g of colorless crystals having a melting point of 101 to 102°C.

^{1}H-NMR($CDCl_3$) δ: 0.87(3H,t,J=7Hz,CH_3), 1.1-1.4(10H,m), 1.30(6H,s,$(CH_3)_2C$), 1.46(2H,m,$NHCH_2\underline{CH_2}$), 1.91(3H,s,CH_3COO^-), 2.30(3H,s,$ArCH_3$), 3.25(2H,br dt-like,$NHCH_2\underline{CH_2}$), 4.43(2H,d,J=

5Hz,$ArCH_2$), 7.07(2H,d,J=8Hz,ArH), 7.15(2H,d,J=8Hz,ArH), 8.18,8.60(each 1H,m,NH), 9.12,9.22(each 1H,m,NH,NH^+).

(10) 4-Octylamino-2-cyclohexylmethylamino-3,6-dihydro-6,6-dimethyl-1,3,5-triazine acetate (the compound of Example 65 in the specification)

100 ml of methanol, 80 ml of acetone and 0.6 ml of concentrated hydrochloric acid were added to 9.0 g (23.5 mmol) of N^1-cyclohexylmethyl-N^5-octyl-biguanide dihydrochloride. The mixture was refluxed for 21 hours, and the solvent was distilled off under reduced pressure. To the residue were added 120 ml of ethanol, 80 ml of water, and 9.5 ml of 5N sodium hydroxide, and the mixture was refluxed for 1 hour, concentrated under reduced pressure, and extracted with methyl ethyl ketone. The extract was washed with water, and 1.7 g of acetic acid was added thereto. The solvent was distilled off under reduced pressure, and the residue was dried well, and recrystallized from methyl ethyl ketone two times to obtain 4.9 g of colorless crystals having a melting point of 70 to 73°C.
^{1}H-NMR($CDCl_3$) δ: 0.88(3H,t,J=7Hz,CH_3), 0.8-1.8(23H,m,cyclohexyl,$(CH_2)_6$), 1.36(6H,s,$(CH_3)_2C$), 1.97(3H,s,CH_3COO^-), 3.16,3.27(each 2H,m,$NHC\underline{H_2}$×2), 8.15(2H,m,NH×2), 9.13(2H,br s,NH,NH^+).

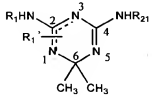
(11) 4-Octylamino-2-(3-quinolylamino)-3,6-dihydro-6,6-dimethyl-1,3,5-triazine acetate (the compound of Example 82 in the specification)

60 ml of ethanol, 40 ml of water and 5.5 ml of 5N sodium hydroxide were added to 4.0 g (8.8 mmol) of 2-amino-4-octylamino-1-(3-quinolyl)-1,6-dihydro-6,6-dimethyl-1,3,5-triazine dihydrochloride (the compound of Example 81). The mixture was refluxed

for 1 hour, concentrated under reduced pressure, and extracted with ethyl acetate. The extract was washed with 10% aqueous sodium acetate, washed with water, and concentrated under reduced pressure to remove the solvent. The residue was recrystallized from methyl ethyl ketone and ether to obtain 2.5 g of pale yellow crystals having a melting point of 62 to 65°C.

^{1}H-NMR($CDCl_3$-D_2O) δ: 0.81(3H,t,J=7Hz,CH_3), 1.1-1.6(12H,m), 1.48(6H,s,$(CH_3)_2C$), 2.06(3H,s,CH_3COO^-), 3.30(2H,t,J= 7Hz,$NHC\underline{H}_2$), 7.4-9.0(6H,m,quinolyl).

The structural formulas of compound Nos. (1) to (11) as prepared above are shown below.



R_1': H attached to the nitrogen atom at position 1 or 3 of the dihydrotriazine ring

Compd. No.	R_1	R_{21}	salt	Ex. No.
(1)	4-methoxybenzyl	decyl	HCl	1
(2)	benzyl	decyl	CH_3SO_3H	2
(3)	4-methoxyphenethyl	decyl	HCl	4
(4)	4-methybenzyl	octyl	(free-form)	37
(5)	4-methoxybenzyl	decyl	CH_3SO_3H	38(2)
(6)	benzyl	nonyl	CH_3CO_2H	48
(7)	benzyl	nonyl	HBr	49
(8)	phenethyl	nonyl	CH_3CO_2H	56
(9)	4-methybenzyl	octyl	CH_3CO_2H	58
(10)	cyclohexylmethyl	octyl	CH_3CO_2H	65
(11)	4-quinolyl	octyl	CH_3CO_2H	82

Experiment 2 Bactericidal activity test

Regarding test compound Nos. (1) to (11) (i.e., compounds of Examples 1, 2, 4, 37, 38 (2), 48, 49, 56, 58, 65 and 82 in the specification) and a control drug (chlorhexidine), bactericidal activity was evaluated using a phenol coefficient measuring method.

As a test bacterium, nine kinds of *S. aureus* 209PJC, MRSA 97-115, MRSA KM 97-53, MRSA KM97-108, VRE 49, *E. coli* NIHJ JC-2, *P. aeruginosa* PAO-1, *P. aeruginosa* No. 12, and *P. aeruginosa* KM97-5 were used; as a medium to be used, a SCD medium (manufactured by Eiken Chemical Co., Ltd. Japan) was used for pre-culture medium; and a heart infusion bouillon medium (manufactured by Eiken Chemical Co., Ltd.) was used as a medium for growing a live bacterium in a test solution after bactericidal treatment. One platinum loop of a test bacterium was inoculated into 20 ml of a medium, and static culture was carried out at 37°C for 18 to 20 hours, and the cells were adjusted with a sterilized physiological saline to 1×10^7/ml, which was used as a test bacterium solution.

Then, a solution of each compound in methanol was subjected to 1/2 stepwise dilution with sterilized water, each 5 ml was dispensed into a test tube, 0.5 ml of the previously prepared test bacterium solution was added thereto, and the mixture was blended well. After 1 minute, 3 minutes and 5 minutes has passed, 5 µl of the test solution was collected and inoculated into 2 ml of a heart infusion bouillon medium, this was cultured at 37°C for 40 to 48 hours, and the presence or absence of growth of a bacterium was determined. A test was performed three times, a minimum concentration at which growth of a bacterium was not recognized two or more times was adopted as a minimal bactericidal concentration (MBC value). A 20% chlorhexidine gluconate solution (manufactured by Wako Pure Chemical Industries, Ltd. Japan) was used as a control drug to perform the similar test.

Test results are shown in the following Tables. Numerical values in Tables represent MBC expressed in µg/ml unit.

Table 1

Bacterium strain	(1)Example 1			(2)Example 2			(3)Example 4		
	1 min	3 min	5 min	1 min	3 min	5 min	1 min	3 min	5 min
S.aureus 209PJC	6.3	3.1	3.1	12.5	6.3	3.1	50	12.5	6.3
MRSA 97-115	25	6.3	6.3	25	12.5	6.3	25	6.3	6.3
E.coli NIHJ JC-2	6.1	3.1	1.6	6.3	3.1	3.1	3.1	1.6	1.6
P.aeruginosa PAO-1	1.6	0.8	0.8	3.1	1.6	1.6	3.1	3.1	1.6

Table 2

Bacterium strain	(4)Example 37			(5)Example 38(2)		
	1 min	3 min	5 min	1 min	3 min	5 min
S.aureus 209PJC	12.5	6.3	6.3	12.5	6.3	3.1
MRSA 97-115	25	25	12.5	25	12.5	12.5
E.coli NIHJ JC-2	12.5	6.3	3.1	6.3	3.1	3.1
P.aeruginosa PAO-1	3.1	3.1	3.1	6.3	3.1	1.6

Table 3

Bacterium strain	(6)Example 48			(7)Example 49			(8)Example 56		
	1 min	3 min	5 min	1 min	3 min	5 min	1 min	3 min	5 min
S.aureus 209PJC	12.5	6.3	6.3	12.5	6.3	6.3	25	12.5	6.3
MRSA 97-115	12.5	6.3	6.3	25	12.5	12.5	25	12.5	6.3
E.coli NIHJ JC-2	6.3	6.3	3.1	12.5	6.3	3.1	12.5	3.1	3.1
P.aeruginosa PAO-1	6.3	3.1	3.1	12.5	3.1	3.1	12.5	3.1	1.6

Table 4

Bacterium strain	(9)Example 58			(10)Example 65		
	1 min	3 min	5 min	1 min	3 min	5 min
S.aureus 209PJC	6.3	3.1	3.1	12.5	3.1	3.1
MRSA 97-115	25	25	12.5	25	12.5	12.5
E.coli NIHJ JC-2	12.5	6.3	6.3	3.1	1.6	1.6
P.aeruginosa PAO-1	12.5	6.3	3.1	3.1	1.6	1.6

Table 5

Bacterium strain	(11)Example 82			Control drug		
	1 min	3 min	5 min	1 min	3 min	5 min
S.aureus 209PJC	12.5	6.3	6.3	62.5	62.5	62.5
MRSA 97-115	50	25	25	1000	250	125
E.coli NIHJ JC-2	6.3	6.3	3.1	62.5	31.3	15.6
P.aeruginosa PAO-1	12.5	12.5	6.3	>400	>400	>400

Table 6

Bacterium strain	(6)Example 48			(9)Example 58		
	1 min	3 min	5 min	1 min	3 min	5 min
MRSA KM 97-53	50	25	25	50	50	25
MRSA KM 97-108	50	50	25	50	50	25
VRE 49	25	12.5	12.5	25	25	12.5
P.aeruginosa No.12	25	12.5	12.5	6.3	6.3	3.1
P.aeruginosa KM 97-5	3.1	3.1	3.1	6.3	3.1	3.1

Table 7

Bacterium strain	Control drug		
	1 min	3 min	5 min
MRSA KM 97-53	500	250	250
MRSA KM 97-108	500	500	500
VRE 49	>1000	>1000	>1000
P.aeruginosa No.12	125	32	32
P.aeruginosa KM 97-5	31	16	8

It is clear that the compounds (1) to (11) have much stronger bactericidal activity than chlorhexidine.

It is declared by the undersigned that all statements made herein of undersigned's own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

This 2 day of June, 2009

Shirou Maeda

Shirou Maeda